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Short report

Study of DXS9895 and DXS7130: Population data from North of Portugal

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ABSTRACT

The study of X-chromosomal short tandem repeats (X-STRs) can complement data obtained with autosomal and Y-STRs. This population study only concerns two X-STRs in order to add complementary data obtained with other X-STRs already studied by our laboratory. DXS9895 and DXS7130 were used to study a population sample of North of Portugal (101 female and 118 male samples). DNA was amplified in a multiplex reaction mix and the automatic detection was performed using capillary electrophoresis. Allele frequencies and several forensic parameters were calculated.

1. Population

A total of 219 healthy and unrelated individuals (101 female and 118 male samples), from North of Portugal.

2. Methods

2.1. DNA extraction and amplification

DNA was extracted from blood samples using the Chelex® method[1] and purified, if necessary (when the sample failed to amplify in PCR reaction), using a modified organic phenol-chloroform-isoamylalcohol method. DXS9895 and DXS7130 were amplified in one multiplex with previously designed primers[2,3] using Qiagen Multiplex kit (Qiagen). PCR was performed in a total volume of 10 μL containing 5 μL of 2× Qiagen Multiplex PCR Master Mix (Qiagen), 1 μL of 10× Primer Mix, 3.5 μL of distilled water and 0.5 μL of template DNA. The final primer concentration in reaction mix was: DXS9895 (0.5 μM) and DXS7130 (4 μM). Thermocycling conditions, using GeneAmp® PCR System 9700 (Applied Biosystems) were: an initial denaturation for 15 min at 95 °C, followed by 10 cycles of 30 sec at 94 °C, 90 sec at 60 °C, 60 sec at 72 °C and 20 cycles of 30 sec at 94 °C, 90 sec at 58 °C, 60 sec at 72 °C and a final extension of 60 min at 60 °C.

Aliquots containing 1 μL of amplified DNA were mixed with 13.5 μL of Hi-Di Formamide (Applied Biosystems) and 0.5 μL of LIZ 500 (Applied Biosystems) as internal standard. After denaturation (5min at 95 °C), samples were injected in ABI PRISM® 3100 Genetic Analyser (Applied Biosystems). Fragment sizes were determined automatically using GeneScan® Analysis Software v3.7 (Applied Biosystems) and genotyping was performed through comparison with DNA control samples 9948 (Promega), K562 (Promega) and 9947A (Applied Biosystems) according to the recommendations of Szibor.[4]

2.2. Data analysis

Allele frequencies, Hardy–Weinberg equilibrium in female samples (exact test) and linkage disequilibrium in male samples were calculated using GENEPOP version 3.4 software package[5] (Table 1). Several parameters of forensic interest were estimated with the formulae proposed by Desmarais[6] (Table 2). Genetic distance estimations based on the number of different alleles (F_{ST}) were calculated using software ARLEQUIN v3.11.[7]

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Table 1
Allele frequencies of DXS9895 and DXS7130 in a population sample of North of Portugal ($n = 218$).

	DXS9895	DXS7130
10	–	0.003
11	–	0.038
12	0.003	0.111
13	0.251	0.035
13.3	–	0.035
14	0.222	0.006
14.3	–	0.190
15	0.346	–
15.2	0.003	–
15.3	–	0.410
16	0.146	–
16.2	0.010	–
16.3	–	0.149
17	0.013	–
17.2	0.006	–
17.3	–	0.016
18.3	–	0.006
p	0.706	0.137

p: Hardy–Weinberg equilibrium exact test in the female sample.

3. Results and other remarks

Allele frequencies are shown in Table 1. The population under study was in Hardy–Weinberg equilibrium and the two X-STR (DXS9895 and DXS7130) were not in linkage disequilibrium ($p = 0.838$). Several forensic parameters are shown in Table 2.

Population differentiation between this population sample (North of Portugal) and Spanish population[8] was evaluated by genetic distance analysis. It was observed that there was no significant genetic distance between these two populations for the two loci under study (DXS9895, $F_{ST} = -0.002$; and DXS7130, $F_{ST} = 0.001$).

This population study concerns only two X-STRs (DXS9895 and DXS7130). However, these two markers used with other X-STRs could be useful in forensic practice, particularly in "deficient paternity" and other kinship cases.

Table 2
Forensic parameters of DXS9895 and DXS7130.

	DXS9895	DXS7130
PIC	0.748	0.759
PD female	0.890	0.906
PD male	0.742	0.750
PE trio	0.703	0.728
PE motherless	0.564	0.595

PIC – polymorphism information content; PD – power of discrimination; PE – power of exclusion.

Conflict of interest
None.

References

1. Walsh PS, Metzer DA, Higuchi R. CHELEX® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 1991; **10**:506–13.
2. Edelmann J, Deichsel D, Hering S, Plate I, Szibor R. Sequence variation and allele nomenclature for the X-linked STRs DXS9895, DXS8378, DXS7132, DXS6800, DXS7133, GATA172D05, DXS7423 and DXS8377. *Forensic Science International* 2002;**129**:99–103.
3. Edelmann J, Szibor R. The X-linked STRs DXS7130 and DXS6803. *Forensic Science International* 2003;**136**:73–5.
4. Szibor R, Edelmann J, Hering S. Cell line DNA typing in forensic genetics: the necessity of reliable standards. *Forensic Science International* 2003;**138**:37–43.
5. Raymond M, Rousset F. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 1995;**86**:248–9.
6. Desmarais D, Zhong Y, Chakraborty R, Perreault C, Busque L. Development of a highly polymorphic STR marker for identity testing purposes at the human androgen receptor gene (HUMARA). *Journal of Forensic Science* 1998;**43**:1046–9.
7. Excoffier L, Lischer HEL. Arlequin suite ver 3.5: a new series of programs to perform population genetic analyses under Linus and Windows. *Molecular Ecology Resources* 2010;**10**(3):564–7.
8. Zarrabeitia MT, Alonso A, Martin J, Gonzalez-Gay MA, Martin-Escudero JC, Pancorbo MM, et al. Study of six X-linked tetranucleotide microsatellites: population data from five Spanish regions. *International Journal of Legal Medicine* 2006;**120**:147–50.